(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 February 2002 (14.02.2002)

PCT

(10) International Publication Number
WO 02/12183 A1

(51) International Patent Classification⁷: C07C 403/24

(21) International Application Number: PCT/KR01/00055

(22) International Filing Date: 15 January 2001 (15.01.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2000/45233 4 August 2000 (04.08.2000) KR

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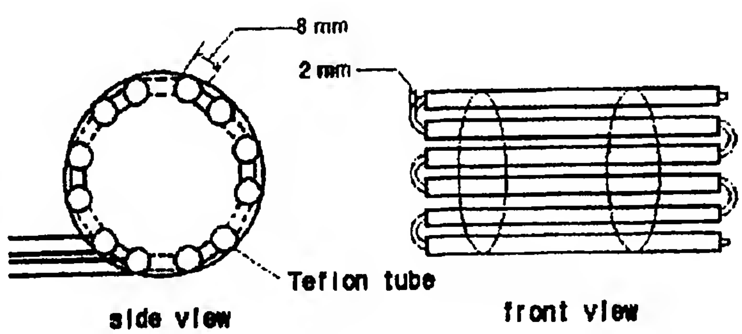
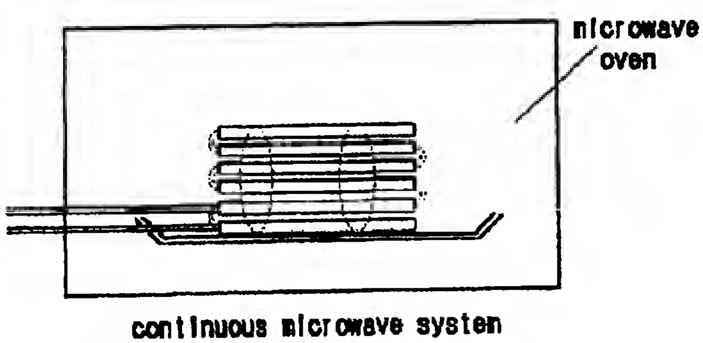
(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO,

[Continued on next page]

(54) Title: PROCESS FOR EXTRACTING ASTAXANTHIN PIGMENT FROM BLUE-GREEN ALGAE AND EXTRACTED PIGMENT THEREOF




(57) Abstract: The present invention relates to a process for extracting astaxanthin pigment from the blue-green algae comprising the steps of i) cultivating the blue-green algae, ii) treating blue-green algae culture suspension with microwave to destroy the cell walls and microbodies, iii) drying it or extracting astaxanthin pigment using the solvent selected from the group consisting of ethanol, methanol, acetone and mixture of them. Further, the present invention also relates to the astaxanthin extracted by above method.

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NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

**(84) Designated States** *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published:**
— *with international search report*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

# PROCESS FOR EXTRACTING ASTAXANTHIN PIGMENT FROM BLUE-GREEN ALGAE AND EXTRACTED PIGMENT THEREOF

## TECHNICAL FIELD

The present invention relates to a process for extracting astaxanthin pigment from the blue-green algae comprising the steps of i) cultivating the blue-green algae, ii) treating blue-green algae culture suspension with microwave to destroy the cell walls and microbodies, iii) drying it or extracting astaxanthin pigment using the solvent selected from the group consisting of ethanol, methanol, acetone and mixture of them. Further, the present invention also relates to the astaxanthin extracted by above method.

More particularly, the present invention relates to a process for extracting astaxanthin pigment from the blue-green algae culture suspension further comprising i) the microwave treatment step, in which the culture suspension is passed through the Teflon tube or is laid on the Teflon extraction vessel for irradiation of microwave 50~1000 watts at the frequency of 900~930 or 2400~2500MHz ; ii) the pigment separation step, in which the obtained pigment is concentrated at reduced pressure using rotary vacuum evaporator. Further, the microwave treatment also provides the sterility of other microorganisms as well as the destruction of cell walls and microbodies in *Haematococcus* species.

## BACKGROUND ART

Astaxanthin (3,3'-dihydroxy-$\beta,\beta'$- carotene-4,4'-dione) is generally obtained from the yeast cell of *Phaffia rhodozyma* (*Phytochemistry*, **15**, 1009, (1976)), blue-green algae of *Haematococcus* species (*Phytochemistry*, **20**, 2561, (1981)) and *Brevibacterium* (*J. general and applied microbiology*, **15**, 127, (1969)). Further, it is chiefly distributed in the sea water animals and fresh water animals (*Phytochemistry* **15**. 1003-1007 (1976)).

Especially, it has been found in the *Crustacea*, such as shrimps or crawfishes ; the tips or yolks of birds ; trouts and salmons as scalet color (*Critical Reviews in Biotechnology* **11(4)**, 297-326(1991)). It contributes to the enhancement of color and flavor, the activation of immunity, the anti-cancer activity by removing oxygen free radical (*Pure & Appl. Chem.*,**51**. 649-660(1979), *J. Korean. Soc. Food Sci. Nutr.* **27(1)**. 163-167(1998)) and the anti-aging metabolism (*Internat. J. Vit. Nutr. Res.* **65**. 79-86(1995)). Further, it can be used as a precursor of vitamin A.

On the other hand, it has been reported that the astaxanthin pigment has an excellent anti-oxidation activity compared to other carotenoid pigment or tocopherol (*Fisheries Sci.*, **62**. 134-140 (1996)). It has been regarded that the astaxanthin pigment has an importance of medical use as well as that of edible pigment (*Crit. Rev. Biotechnol.* **11**. 297-326 (1991)).

However, the extraction of astaxanthin from the *Crustacea*, such as shrimps or crawfishes has been hardly tried, because only a small amount of astaxanthin is contained. Further, the extraction of astaxanthin from a *Phaffia rhodozyma* has been hardly applied either, since

the cell walls of such microorganisms are too hard to extract astaxanthin from them.

Until now, the extraction of astaxanthin from *Haematococcus* species has not been developed in the commercially available scale either, since the growth of *Haematococcus* species is not satisfactory for cultivation and the contamination is often occurred during the cultivation.

In order to obtain the astaxanthin from *Haematococcus* species, the development of efficient method for destroying the cell walls of *Haematococcus* species is necessarily required. Sometimes, to avoid the elevation of the cost, the dried astaxanthin powder without destroying the cell walls has been used (*J. Applied phycology* **4**, 267-279(1992)). However, the astaxanthin powder manufactured without such treatment shows very low bioavailability, when it is used as animal feed or food additive.

Therefore, a number of methods for destroying cell walls of *Haematococcus* species have been developed.

1) A mechanical method comprising i) freezing dried *Haematococcus* species using liquid nitrogen, ii) destroying cell walls using high speed impact mill or jet mill, and iii) obtaining astaxanthin has been disclosed in U.S. Pat. No.4,871,551 and No.6,022,701.

2) A physical method comprising i) destroying cell walls using French pressure or Braun homogenizers, and ii) extracting astaxanthin with solvent has been disclosed in *Methods in enzymology* **213**, 386-391(1992); *J. Agric. Food Chem.*, **46**, 3371-3375(1998); U.S. Pat. No.5,744,502,.

3) A biochemical method comprising the step destroying cell walls using digestion enzyme, such as cellulase, hemicellulase and pectinase has been disclosed in *Appl. and Environ. Microbiol.* **35(6)**. 1155-1159(1978).

4) Another biochemical method comprising i) treating protease in order to digest the proteins in cell walls, and ii) applying osmotic pressure to cell walls has been disclosed in Japanese laid open patent publication No.1993-68585.

However, any of above disclosed methods can not afford the commercially available extracting method for astaxanthin, due to the destruction of astaxanthin pigment during the process and the low yield of astaxanthin. Further, the direct extraction method of astaxanthin using the solvent, such as ethanol or acetone, also cannot be commercialized due to its high cost and low yield.

To solve above problems, the inventors developed a microwave treatment method to destroy cell walls for efficient extraction of pigment and also designed the vessel and the tube for microwave irradiation. Therefore, the inventors accomplished effective and cost efficient method for obtaining astaxanthin pigment.

## DISCLOSURE OF INVENTION

The object of the present invention is to provide a process for extracting astaxanthin pigment from the blue-green algae comprising the

steps of i) cultivating the blue-green algae, ii) suspending cultivated blue-green algae with water iii) treating culture suspension with microwave to destroy the cell walls and microbodies, and iv) drying obtained material containing astaxanthin pigment.

The another object of the present invention is to provide a process for extracting astaxanthin pigment from the blue-green algae comprising the steps of i) cultivating the blue-green algae, ii) suspending cultivated blue-green algae with water iii) treating culture suspension with microwave to destroy the cell walls and microbodies, and iv) extracting astaxanthin pigment using the solvent selected from the group consisting of ethanol, methanol, acetone and mixture of them.

The blue-green algae used in the present invention is at least one selected from the group consisting of *Acetabularia mediterranea, Chlamydomonas nivalis, Euglena rubida, Haematococcus pluvialus, Haematococcus lacustris* and *Haematococcus droeabicensis.*

The microwave treatment is selected from the continuous process in which the culture suspension is passed through Teflon tube, or the fixed process in which the culture suspension is laid on the Teflon extraction vessel, for irradiation of microwave 50~1000 watts at the frequency of 900~930 or 2400~2500MHz.

The present invention also provide a process for extracting astaxanthin pigment from the blue-green algae, further comprising the pigment separation step, in which the obtained pigment is concentrated at reduced pressure using rotary vacuum evaporator.

The yield of astaxanthin pigment is 5~95 wt% from total

carotenoid contained in blue-green algae, and the purity of astaxanthin pigment is 50~95 wt% of obtained material.

The amount of extraction solvent is 5~20 vol. part compared to 1 vol. part of suspension, or 5~10 (vol./wt) part compared to 1 wt part of dried content of blue-green algae.

The further object of the present invention is to provide a method for using astaxanthin pigment as cosmetics, animal feeds or food additives.

## BRIEF DESCRIPTION OF DRAWINGS

FIG 1 shows a schematic view of continuous microwave treatment system using the Teflon tube to the suspension according to the present invention.

FIG 2 shows a schematic view of fixed microwave treatment system using the Teflon extraction vessel to the suspension according to the present invention.

FIG 3 shows an optical microscope (×400) photo of *Haematococcus* species (green) without microwave treatment (A); *Haematococcus* species (red) without microwave treatment (B); and *Haematococcus* species with microwave treatment (C) after cultivating it according to the present invention.

FIG 4 shows HPLC data of obtained extract followed by i)

cultivation of *Haematococcus* species ii) microwave treatment, and iii) ethanol extraction according to the present invention.

## BEST MODE FOR CARRYING OUT THE INVENTION

The principle of microwave treatment can be explained as follows.

When the microwaves are irradiated into the *Haematococcus* species according to the methods shown in FIG 1 or FIG 2, the free water and other dipoles in the cells are rotated according to the electric field alternation, which converts microwave energy into thermal energy. Then, the cell walls and microbodies (nucleus, mitochondria, Golgi apparatus) in the cells are destroyed according to the elevation of internal pressure followed by internal heating. Therefore, the microwave treatment enables the extraction of pigment without physical destruction of cell walls. Further, the extraction by organic solvent can be easily accomplished by the microwave treatment, because organic solvent can be easily diffused into the cells.

Therefore, the inventors adopt a microwave treatment method and apply it to the extraction of astaxanthin pigment by modifying the methods disclosed in *Trends in analytical chemistry* **13(4)**. 176-184(1994); *Anal. Chem.* **66**. 1097-1106(1994); U.S. Pat. No.5,002,784; U.S. Pat. No.5,458,897.

Further, the inventors design the microwave treatment systems for extracting astaxanthin effectively. One is microwave treatment system using the Teflon tube as shown in FIG 1 and another is fixed microwave

treatment system using the Teflon extraction vessel as shown in FIG 2.

As shown in FIG 1, Teflon tube is inserted into the microwave oven and the length and diameter of Teflon tube are designed according to the microwave holding time and microwave treatment capacity. The cultivated suspension is passed through the tube using Variable speed peristaltic pump (Model : AS-90361, Won Corporation, Korea).

As shown in FIG 2, Teflon extraction vessel is laid on the center of microwave oven and the amount of cultivated suspension is put on the vessel in microwave oven. In this fixed system, Teflon tube and pump are not required.

The output of microwave is in the range of 50~1,000 watts at the frequency of 916 or 2450MHz. The microwaves are irradiated into Teflon extraction vessel or Teflon tube for 10~500 seconds.

The morphology of *Haematococcus* species applied to this system is as follows. They are 10~50μm size of plankton belonging to blue-green algae, and growing in the fresh water having two flagella. Further, their cell walls are thick and composed of sugar protein unlike other algaes or higher plants. Photosynthesis is carried out on aerobic condition.

*Acetabularia mediterranea, Chlamydomonas nivalis, Euglena rubida* and *Haematococcus* species (*Haematococcus pluvialus flotow* and *Haematococcus lacustris*) are obtained from CCAG; Gottingen, CCAP; UK, CCAT; USA, NIES; Tsukuba Japan, SCCAP; Denmark, UTEX; USA.

Among them, *Haematococcus* species producing astaxanthin more than other microorganisms are cultivated using following media under

following growth conditions.

The preferred composition of medium is 14.6mM sodium acetate, 2.7mM L-asparagine, 2.0g/L yeast extract, 0.985mM $MgCl_2{\cdot}6H_2O$, 0.036mM $FeSO_4{\cdot}7H_2O$, 0.135mM $CaCl_2{\cdot}2H_2O$ in the sterile water at pH 6.5~8.0, preferably 6.8.

In the cultivation medium, the air is inserted with 1.5% carbon dioxide. The light is illuminated by white fluorescent lamp on 20µ $Em^{-2}S^{-1}$ at 18～28℃, preferably 25℃. 45mM of sodium acetate and 450 µM of $FeSO_4{\cdot}7H_2O$ are added under 120µ$Em^{-2}S^{-1}$ illumination at 18~28℃, preferably 20℃ for 4 days. Grown *Haematococcus* species become red from initial green color.

The cultivation of *Haematococcus* species has been carried out until the average diameter of cells becomes 20～80㎛, cell density becomes $10^6$~$10^7$/ml and the dried weight of cells becomes 0.5~3.0g/L on 5L fermenter. The cells are obtained by continuous flow centrifuges. Then, obtained cells are laid on microwave illumination and other conditions for extracting astaxanthin, such as extraction solvent, volume of solvent, extraction time and temperature are measured to afford optimal extraction.

The present invention will be more specifically explained by the following examples. However, it should be understood that the examples are intended to illustrate but not in any manner to limit the scope of the present invention.

## EXAMPLES

(Example 1) Pigment extraction yield test according to continuous or fixed microwave treatment system

The extraction yield of astaxanthin was measured under microwave illumination according to the conditions; microwave output 50~1,000 watts, at frequency of 916 or 2450㎒, for 10~500 seconds. Cultivated cells were treated with microwave by ⅰ) continuous microwave treatment system using the Teflon tube as shown in FIG 1 and ⅱ) fixed microwave treatment system using the Teflon extraction vessel as shown in FIG 2. 10 vol. part of ethanol was added to 1 vol. part of cultivated suspension. After extraction at 40℃ for 24 hours, the extract was laid at -20℃ for more than 30 minutes to remove the lipid. After centrifuging obtained extract using centrifuger (10,000 X g), the astaxanthin extract was obtained by concentrating the supernatant at reduced pressure. To measure the extraction yield of pigment, absorbance was measured adding acetone by UV/VIS Spectrophotometer Biochrom 4060, Pharmacia, German at 478nm.

Control group was prepared after removing chlorophyll from the cells. Following is a method for preparing control group. After concentrating cultivated cells using centrifuger (3500 X g) for 5 minutes, the concentrated cells were dissolved with 30% of methanol solution adding 5% of potassium hydroxide. After heating the solution at 70℃ for 5 minutes, the solution was centrifuged, and the supernatant was removed. To the residue, 3~5 drops of acetic acid were added and 1ml of preheated (55℃) stock solution of dimethylsulfoxide (DMSO) was added. Then, the mixture was vigorously agitated in order to fully destroy the algae cells. To extract the pigment, 1ml of acetone, 1ml of

petroleum ether and 1ml of sodium chloride (20%) were added in this order. After storaging in the refrigerator, the solution was centrifuged at 10,000 X g for 5 minutes. After obtaining petroleum ether layer in which the pigment was dissolved, the petroleum ether was evaporated at reduced pressure. 1ml of acetone was added to obtained material and the absorbance was measured at 478nm for confirming the pigment extract.

The pigment extraction yield was calculated by following equation.

Pigment extraction yield (%) = $B/A \times 100$

A : the absorbance of control group after destroying cell walls using DMSO at 478nm;

B : the absorbance of experimental group after irradiation of microwave and extraction with organic solvents at 478nm

Table 1 shows the result of pigment extraction yield according to the variation of frequency, time and output. All results are mean values of three times experiments.

Table 1.

| Frequency (MHz) | Irradiation time (sec) | Output (watt) | | |
|---|---|---|---|---|
| | | 50 | 500 | 1,000 |
| 916 | 10 | 3 | 3 | 4 |
| | 30 | 28 | 43 | 42 |
| | 60 | 54 | 89 | 83 |
| | 120 | 57 | 87 | 80 |
| | 240 | 49 | 75 | 76 |
| | 500 | 48 | 71 | 69 |

| | | | | |
|---|---|---|---|---|
| 2450 | 10 | 2 | 3 | 4 |
| | 30 | 26 | 47 | 46 |
| | 60 | 59 | 95 | 94 |
| | 120 | 61 | 92 | 89 |
| | 240 | 47 | 82 | 85 |
| | 500 | 54 | 74 | 75 |

unit : %

(Example 2) Pigment extraction yield test according to extraction solvent and time after microwave irradiation

According to the method in Example 1, frequency 2450MHz; output 500 watts; and irradiation time 60 seconds were adjusted for microwave treatment. The amount of extraction solvent was 10 vol. part compared to 1 vol. part of cultivated suspension. The extraction temperature was at 40℃. The astaxanthin extract was obtained by using rotary vacuum evaporator in the dark room. After adding acetone, the absorbance of pigment extract was measured at 478nm. Finally, pigment extraction yield was measured using UV/VIS Spectrophotometer Biochrom 4060.

Table 2 shows the result of pigment extraction yield according to the variation of solvent and time. All results are mean values of three times experiments.

Table 2.

| Extraction solvent | Extraction time (hour) | | | |
|---|---|---|---|---|
| | 0 | 6 | 12 | 24 |
| Control | 0 | 1 | 2 | 2 |
| Ethanol | 2 | 45 | 87 | 95 |
| Methanol | 3 | 35 | 67 | 80 |
| Acetone | 2 | 21 | 52 | 65 |

unit : %

* Control group shows the absorbance to the cultivated suspension without microwave treatment

(Example 3) Pigment extraction yield test according to extraction temperature and time after microwave irradiation

The experiment was carried out as the same manner of Example 2 except that the extraction temperatures were varied. Finally, pigment extraction yield was measured using UV/VIS Spectrophotometer Biochrom 4060.

Table 3 shows the result of pigment extraction yield according to the variation of extraction temperature and time. All results are mean values of three times experiments.

Table 3.

| Extraction temperature | Extraction time (hour) | | | |
|---|---|---|---|---|
| | 0 | 6 | 12 | 24 |
| 20 | 4 | 11 | 35 | 59 |
| 40 | 5 | 45 | 87 | 95 |
| 80 | 5 | 39 | 73 | 80 |

unit : %

(Example 4) Pigment extraction yield test according to extraction solvent volume after microwave irradiation

The experiment was carried out as the same manner of Example 2 except that the extraction solvent volumes were varied. The extraction time was 24 hours and extraction temperature was 40℃. Finally, pigment extraction yield was measured using UV/VIS Spectrophotometer Biochrom 4060.

Table 4 shows the result of pigment extraction yield according to the variation of extraction solvent volume. All results are mean values of three times experiments.

Table 4.

| Ethanol : Suspension (V/V) | Extraction yield (%) |
|---|---|
| 1 : 1 | 6 |
| 5 : 1 | 68 |
| 10 : 1 | 95 |

unit : %

(Example 5) Analysis for measuring the astaxanthin contents in extracted pigment

The total carotenoid contents were measured after removing chlorophyll from the cells. After concentrating 1ml of cultivated cells using centrifuger (3500 X g) for 5 minutes, the concentrated cells were dissolved with 30% of methanol solution adding 5% of potassium hydroxide. After heating the solution at 70℃ for 5 minutes, the

solution was centrifuged, and the supernatant was removed. To the residue, sterile water was added twice for washing. Then, 3~5 drops of acetic acid were added and 1ml of preheated (55℃) stock solution of dimethylsulfoxide (DMSO) was added. Then, the mixture was vigorously agitated in order to fully destroy the algae cells.

To extract the pigment, 1ml of acetone, 1ml of petroleum ether and 1ml of sodium chloride (20%) were added in this order. After storaging in the refrigerator, the solution was centrifuged at 10,000 X g for 5 minutes. The absorbance of the supernatant was measured at 474nm.

Using 1ml of pigment extract solution in Example 4, the solvent was evaporated at reduced pressure. 1ml of petroleum ether was added and the absorbance was measured at 474nm. The extraction yield of pigment was measured by comparing the amount of pigment extracted by dimethylsulfoxide (100%).

Total carotenoid contents were measured by using following equation which contains 1% extinction coefficient (2,100) and dried content of cells (*Appl. and Environ. Microbiol.*, **55**. 116-124(1989)). From ethanol extract, the contents of astaxanthin pigment was measured after evaporating extraction solvent at vacuum and reduced pressure. Then, obtained astaxanthin pigment was dissolved in chloroform before analyzing HPLC (Waters 486, USA). The HPLC data was shown in FIG 4. Detailed analytical conditions were i) mobile phase = n-hexane/acetone (8:2); ii) stationary phase = silica (4.0 X 250㎜); iii) solvent = chloroform; iv) flow rate = 1ml/min, v) wave length = 476nm; vi) standard = astaxanthin (Sigma, 98% up) dissolved in chloroform. Then, the contents of astaxanthin were measured according

to the standard quantification curve which was prepared by the measurement of standard concentration of astaxanthin.

Total carotenoid contents (mg/g algae cell dry weight) = (A x M x 100)/(21 x D)

A : absorbance of pigment at 474nm;

M : amount of solvent used for extraction (ml);

D : dry weight of *Haematococcus* species cells;

21 : 1% of extinction coefficient from the weight of cells = 2,100

The contents of total carotenoid after microwave irradiation using ethanol solvent and the contents of astaxanthin were measured. The result as shown in Table 5 was mean values of three times experiments.

Table 5.

| Extraction method / Cells | Pigment extraction after DMSO destruction | | Pigment extraction after microwave irradiation | | |
|---|---|---|---|---|---|
| | Total carotenoid (mg/g yeast) | Extraction yield (%) | Total carotenoid (mg/g yeast) | Extraction yield (%) | Astaxanthin (mg/ml) |
| *Haematococcus* species | 48.01 | 100 | 45.61 | 95 | 43.33 |

* The astaxanthin content was measured from the extract after irradiation microwave

(Example 6) Microorganism sterilization test according to the kind of microorganism and irradiation time of microwave

To measure the sterilization effect by microwave irradiation, the

test was carried out after irradiation of microwave output 50~1,000 watts, at frequency of 2450MHz, for 10~200 seconds. The microorganisms used in this Example were *Haematococcus* species, *E. coli*, yeast, and fungus. The cultivation medium of *Haematococcus* species comprises 14.6mM of Sodium acetate, 2.7mM of L-asparagine, 2.0g/L of yeast extract, 0.985mM of $MgCl_2 \cdot 6H_2O$, 0.036mM of $FeSO_4 \cdot 7H_2O$, 0.135mM of $CaCl_2 \cdot 2H_2O$, 1.5% of agar medium in the sterile water. The microorganisms were spread in the cultivation medium and cultivated for 4 days. The test was measured by eyes after cultivating *E. coli* in the Desoxycholate lactose agar medium; cultivating bacteria and yeast in the YM (yeast malto) medium; cultivated fungus in the PDA (Potato dextrose agar) medium for 3 or 4 days.

After irradiation of microwave, the number of growing microorganisms was measured in three times. The Table 6 showed the results.

Table 6.

| Frequency (MHz) | Irradiation time (sec) | Output (watt) | | |
|---|---|---|---|---|
| | | 50 | 500 | 1,000 |
| *Haematococcus* species | 0 | +++ | | |
| | 10 | +++ | + | + |
| | 30 | ++ | - | - |
| | 60 | + | - | - |
| | 120 | - | - | - |
| | 180 | - | - | - |
| E. *coli* and Yeast | 0 | + | | |
| | 10 | + | - | - |
| | 30 | - | - | - |
| | 60 | - | - | - |
| | 120 | - | - | - |
| | 180 | - | - | - |
| Bacteria | 0 | + | | |
| | 10 | + | - | - |
| | 30 | - | - | - |
| | 60 | - | - | - |
| | 120 | - | - | - |
| | 180 | - | - | - |
| Fungus | 0 | + | | |
| | 10 | + | - | - |
| | 30 | - | - | - |
| | 60 | - | - | - |
| | 120 | - | - | - |
| | 180 | - | - | - |

+++ : so many colonies ++ : more than 10 colonies
\+ : more than 1 colony - : not detected

(Reference Example) The production of *Haematococcus* species dried product

The example of producing *Haematococcus* species dried product was as follows.

After irradiating microwaves to the cultivated *Haematococcus* species suspension as the same manner in Example 2, the cells were obtained after continuous flow centrifuges (7,000 X g). The obtained cells were washed twice using clean water and the content of water made to be less than 10%. Using spray dryers, vacuum drum dryers or trays dryers, the *Haematococcus* species dried product was manufactured.

WHAT IS CLAIMED IS :

1. A process for extracting astaxanthin pigment from the blue-green algae comprising the steps of i) cultivating the blue-green algae, ii) suspending cultivated blue-green algae with water iii) treating culture suspension with microwave to destroy the cell walls and microbodies, and iv) drying obtained material containing astaxanthin pigment.

2. A process for extracting astaxanthin pigment from the blue-green algae comprising the steps of i) cultivating the blue-green algae, ii) suspending cultivated blue-green algae with water iii) treating culture suspension with microwave to destroy the cell walls and microbodies, and iv) extracting astaxanthin pigment using the solvent selected from the group consisting of ethanol, methanol, acetone and mixture of them.

3. The process for extracting astaxanthin pigment from the blue-green algae according to claim 1 or claim 2, wherein said blue-green algae is at least one selected from the group consisting of *Acetabularia mediterranea, Chlamydomonas nivalis, Euglena rubida, Haematococcus pluvialus, Haematococcus lacustris* and *Haematococcus droeabicensis.*

4. The process for extracting astaxanthin pigment from the blue-green algae according to claim 1 or claim 2, wherein the microwave treatment is selected from the continuous process in which the culture suspension is passed through Teflon tube, or the fixed process in which the culture suspension is laid on the Teflon extraction vessel, for irradiation of microwave 50～1000 watts at the frequency of 900～930 or 2400～2500㎒.

5. The process for extracting astaxanthin pigment from the blue-green algae according to claim 2, further comprising the pigment separation step, in which the obtained pigment is concentrated at reduced pressure using rotary vacuum evaporator.

6. The process for extracting astaxanthin pigment from the blue-green algae according to claim 1 or claim 2, wherein the yield of astaxanthin pigment is 5~95 wt% from total carotenoid contained in blue-green algae, and the purity of astaxanthin pigment is 50~95 wt% of obtained material.

7. The process for extracting astaxanthin pigment from the blue-green algae according to claim 1 or claim 2, wherein the amount of extraction solvent is 5~20 vol part compared to 1 vol part of suspension, or 5~10 (vol/wt) part compared to 1 wt part of dried content of blue-green algae.

8. Astaxanthin pigment extracted from the blue-green algae according to the method in claim 1 or claim 2.

9. A method for using astaxanthin pigment as cosmetics, animal feeds or food additives.

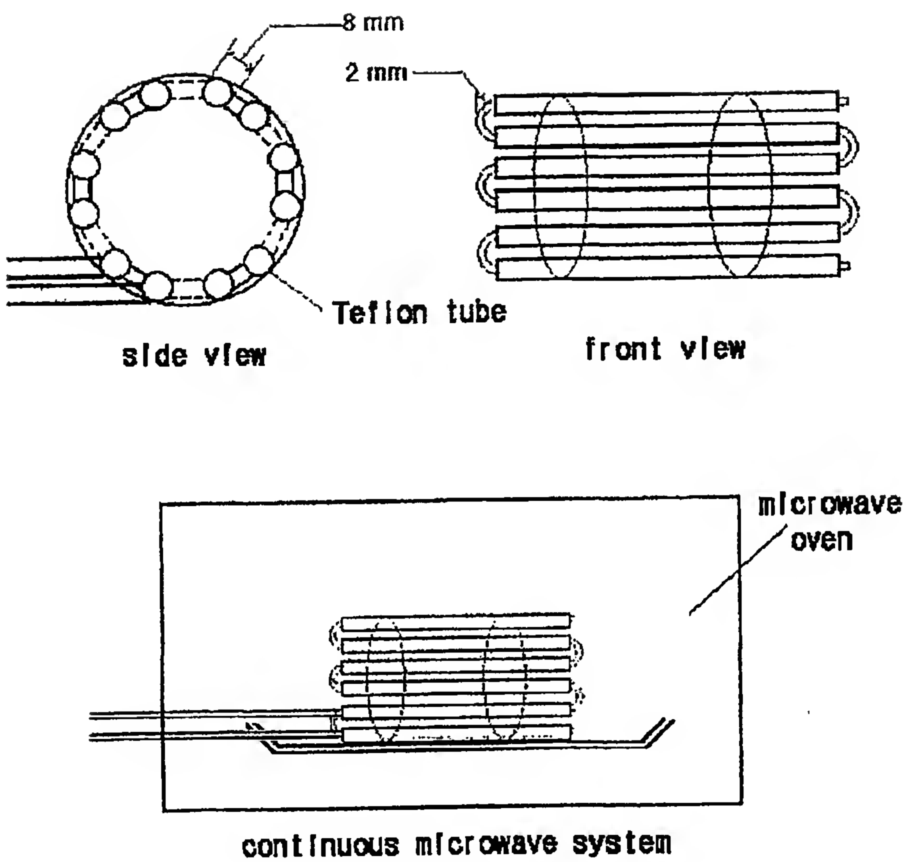

8 mm
2 mm
Teflon tube
side view
front view
microwave
oven
continuous microwave system

FIG 1

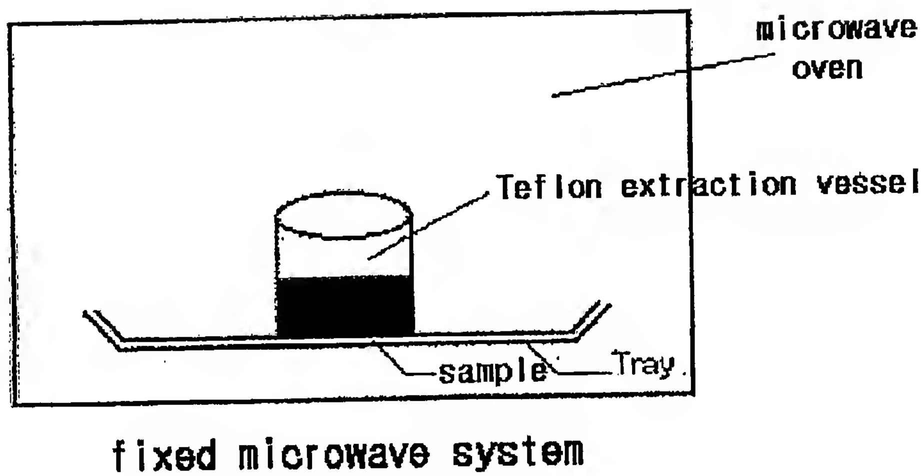
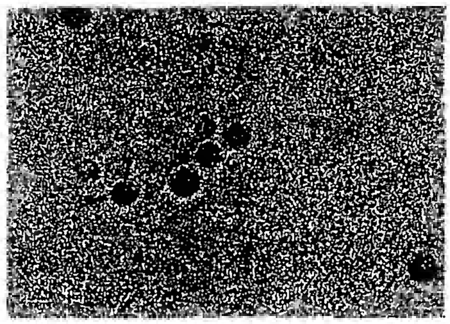
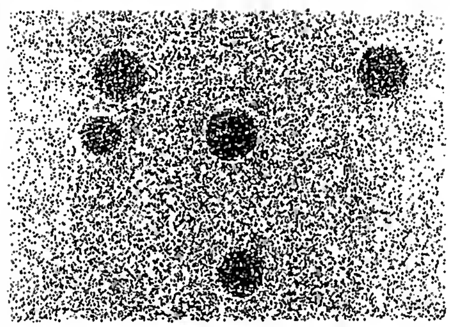

microwave oven
Teflon extraction vessel
sample
Tray
fixed microwave system

FIG 2

(A)



(B)



(C)

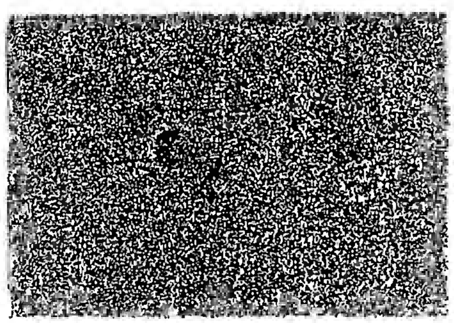
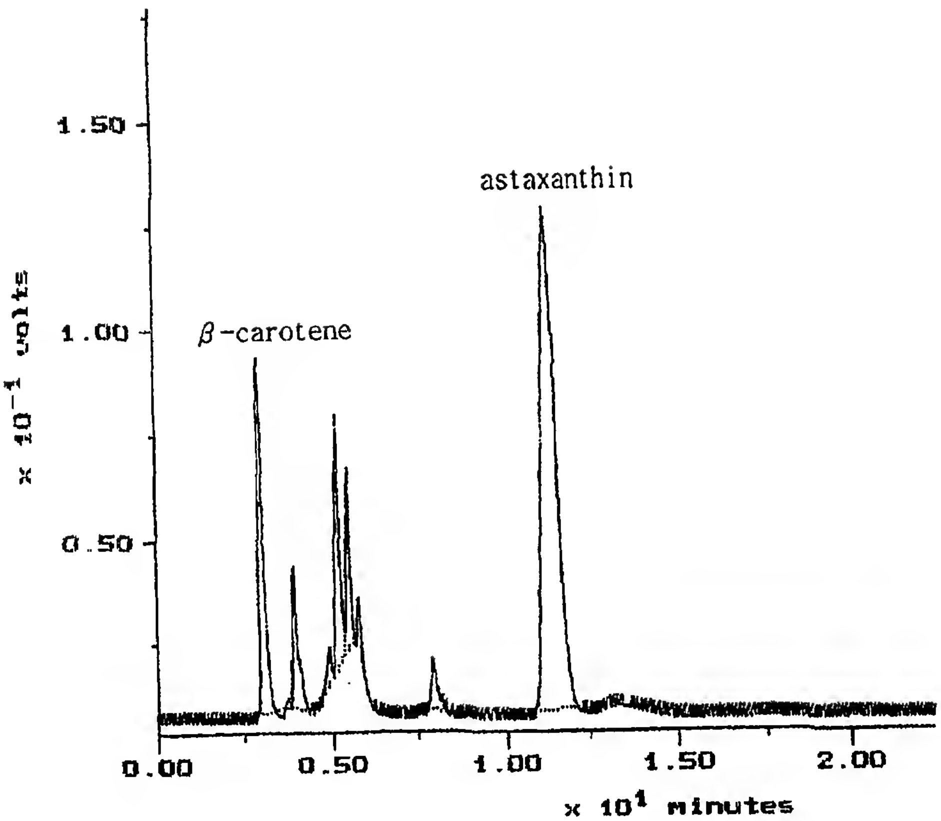


FIG 3



1.50
1.00
0.50
x 10⁻¹ volts
β-carotene
astaxanthin
0.00
0.50
1.00
1.50
2.00
x 10¹ minutes


FIG 4

## INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 01/00055

CLASSIFICATION OF SUBJECT MATTER

IPC[7]: C07C 403/24

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC[7]: C07C 403/24

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN:CAS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
„A" document defining the general state of the art which is not considered to be of particular relevance
„E" earlier application or patent but published on or after the international filing date
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„O" document referring to an oral disclosure, use, exhibition or other means
„P" document published prior to the international filing date but later than the priority date claimed

„T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
„X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
„Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
„&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 27 June 2001 (27.06.2001) | 18 July 2001 (18.07.2001) |
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## INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 01/00055

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